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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/631,224	07/28/2003	Cheng J. Cao	DAM 581-02	3741

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US ARMY SOLDIER AND BIOLOGICAL CHEMICAL COMMAND
OFFICE OF THE CHIEF COUNSEL/IP TEAM (BLDG E4435)
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APG, MD 21010-5424

EXAMINER	
SHAHNAN SHAH, KHATOL S	

ART UNIT	PAPER NUMBER
1645	

MAIL DATE	DELIVERY MODE
08/23/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/631,224	CAO ET AL.	
	Examiner	Art Unit	
	Khatol S. Shahnan-Shah	1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 June 2007*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-9 and 14-18* is/are pending in the application.

4a) Of the above claim(s) *1-9,14,17 and 18* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *15* is/are rejected.

7)☒ Claim(s) *16* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

RESPONSE TO AMENDMENT

1. The amendment and response filed 06/08/2007 have been entered into the record. Claims 15-16 have been amended. A substitute specification has been submitted. The reference numerals, which appeared on the left hand side of all pages of the original specification, have been removed. No other changes were made to the specification as originally filed.

Status of the Claims

2. Claims 1-9 and 14-18 are pending. Claims 10-13 and 19-22 have been cancelled by a previous amendment. Claims 15-16 are under examination. Claims 1-9, 14 and 17-18 are withdrawn from consideration as being drawn to non-elected inventions.

3. The text of Title 35 of the U.S. Code not reiterated herein can be found in the previous office action.

Objections Withdrawn

4. Objection to the specification made in paragraph 3 of the office action mailed January 9, 2007 has been withdrawn in view of applicants' amendments of 6/08/2007.

5. Objection to claims 15-16 made in paragraph 6 of the office action mailed January 9, 2007 has been withdrawn in view of applicants' amendments of 6/08/2007.

Rejections Withdrawn

6. Rejection of claims 15-16 under 35 U.S.C. 112 second paragraph made in paragraph 8 of the office action mailed January 9, 2007 has been withdrawn in view of applicants' amendments of 6/08/2007.

Rejections Maintained

7. Rejection of claim 15 under 35 U.S.C. 103 (a) made in paragraph 11 of the office action mailed January 9, 2007 is maintained.

The rejection was as recited below:

Claim 15 is rejected under 35 U.S.C. 103(a) as being unpatentable over Letertre et al. (Molecular and Cellular Probes, vol.17, pp. 139-147, 2003) in light of O'Connell et al. (23rd Army Science Conference, December 2002), and further in view of Borst et al.

(Infection and Immunity vol. 61, no. 12, pp. 5421-5425, 1993 and sequence alignment # STAENAB).

Claim 15 is drawn to a method of determining the presence of staphylococcal enterotoxin A gene in a sample, comprising:

contacting a target nucleic acid sequence forming at least a portion of a nucleic acid encoding staphylococcal enterotoxin A, with polymerase chain reaction reagents specific for the target nucleic acid sequence, the polymerase chain reaction reagents including a primer selected from the group consisting of a forward primer having a specific sequence selected from the group consisting of [SEQ ID NO: 3], [SEQ ID NO: 4] and combinations thereof and a reverse primer having a specific sequence selected from the group consisting of [SEQ ID NO: 5], [SEQ ID NO: 6] and combinations thereof, a polymerase enzyme, and a nucleic acid probe, wherein the nucleic acid probe further comprises:

a nucleic acid sequence that hybridizes to a portion of the target nucleic acid sequence wherein the portion is unique to the nucleic acid encoding staphylococcal enterotoxin A;

a reporter attached to a 5' end of the nucleic acid probe, said reporter capable of omitting a detectable signal;

a quencher attached to a 3' end of the nucleic acid probe capable of substantially quenching the reporter and prevent omission of the detectable signal, when the nucleic acid probe is intact, wherein the reporter becomes substantially unquenched when the nucleic acid probe is cleaved by the polymerase enzyme during amplification of the target nucleic acid sequence;

amplifying the target nucleic acid sequence by thermal cycling, wherein the thermal cycling is sufficient to amplify the target nucleic acid sequence; and

measuring the level of fluorescence in the sample subsequent to thermal cycling, and further wherein the level of detectable signal is correlated to an amount of the nucleic acid encoding *staphylococcal* enterotoxin A in the sample, thereby quantitatively detecting the nucleic acid encoding *staphylococcal* enterotoxin A in the sample.

Letertre et al. teach a method of determining the presence of *staphylococcal* enterotoxin A gene in a sample using Real-Time Fluorogenic Polymerase Chain Reaction (PCR), see title and abstract. Letertre et al. teach the step of contacting a target nucleic acid sequence forming at least a portion of a nucleic acid encoding *staphylococcal* enterotoxin A, with polymerase chain reaction reagents specific for the target nucleic acid sequence, the polymerase chain reaction reagents including forward and reverse primers (see page 140, selection of primers and tables 1-3). Letertre et al. teach a nucleic acid sequence that hybridizes to a portion of the target nucleic acid sequence wherein the portion is unique to the nucleic acid encoding staphylococcal enterotoxin A (see page 140, selection of primers and tables 1-3). Letertre et al. teach a set of universal primers, FastStart Taq DNA polymerase, lighter cycle system from Roche Diagnostics and FastStart DNA Master SYBER Green in a Real –Time fluorescence PCR which covers the limitations (such a reporter attached to a 5' end of the nucleic acid probe, said reporter capable of omitting a detectable signal; and a quencher attached to a 3' end of the nucleic acid probe capable of substantially quenching the reporter and prevent omission of the detectable signal, when the nucleic acid probe is intact, wherein the reporter becomes substantially unquenched when the nucleic acid probe is cleaved by the polymerase enzyme during amplification of the target nucleic acid sequence), see page 143. Letertre et al. teach amplifying the target nucleic acid sequence by thermal cycling, wherein the thermal cycling is sufficient to amplify the target nucleic acid sequence (see page 142). Letertre et al. also teach measuring the level of fluorescence in the sample subsequent to thermal cycling, and further wherein the level of detectable signal is correlated to an amount of the nucleic acid encoding staphylococcal enterotoxin A in the sample, thereby quantitatively detecting the nucleic acid encoding staphylococcal enterotoxin A in the sample (see page 143). Letertre et al. do not specifically teach sequences such as SEQ ID Nos: 3, 4, 5 or 6. These deficiencies have been overcome by the teach of Borst et al. (Infection and Immunity vol. 61, no. 12, pp. 5421-5425, 1993 and sequence alignment # STAENAB).

Borst et al. teach primers consisting of sequences from *Staphylococcus aureus* enterotoxin A gene 100% identical to SEQ ID NO: 3 and SEQ ID NO: 5. (see page 5423 and sequence alignment # STAENAB for SEQ ID NO: 3 and NO: 5).

It would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine the teaching of Letertre et al. and Borst et al. to obtain a method of determining the presence of staphylococcal enterotoxin A gene in a sample because Letertre et al. teach a method of determining the presence of *staphylococcal* enterotoxin A gene in a sample using a Real –Time fluorescence PCR One of ordinary skill in the art would have been motivated to use the sequences taught by Borst et al. as forward and reverse primers because these primers are the nucleic acids from a portion of nucleic acid encoding *staphylococcal* enterotoxin A . One would have been motivated to use the already known method and primers of the prior art to detect the presence of staphylococcal toxin in a sample.

Note: It is noted that Real –Time fluorescence PCR works similarly as evidenced by O'Connell et al below:

O'Connell et al. (23rd Army Science Conference, December 2002) teach " Real-time fluorescent PCR" process steps that involve the addition of a third small fragment of DNA to the reaction mixture. The DNA/RNA detection reaction combines standard PCR with a third reagent, a probe DNA molecule that hybridizes to a target sequence between the sequences bound by the two PCR primers. The probe is labeled at one end with a fluorescent dye molecule and at the other end with a molecule that quenches the fluorescence of the dye molecule, such that the proximity of these two molecules results in a quenching of the dye's fluorescence. When a thermostable DNA polymerase extends one of the two primers into the area where the probe is bound, the 5' nuclease activity of Taq DNA polymerase degrades the probe and releases the fluorescent and quencher molecules bound to the probe ends. The separation of the dye and the quencher results in an increase in the overall fluorescence of the sample mixture. A detector in the PCR instrument continually monitors and records the fluorescence present in the sample. Significant accumulation of fluorescence in the sample above background level indicates a positive detection of the target DNA.

Applicants' arguments filed 06/08/2007 have been fully considered but they are not persuasive.

Applicants argue:

Claim 15 was rejected under 35 U.S.C. §103(a) as being unpatentable over the publications of Letertre et al. in light of O'Connell et al., and further in view of Borst et al. Letertre and O'Connell were relied on to teach the method steps of real-time fluorescence PCR, while Borst was relied on to teach primers "100% identical to SEQ ID NO: 3 and SEQ ID NO: 5." It is respectfully submitted that this argument is untenable and should be withdrawn because Borst does not actually teach primers identical to those disclosed and 'claimed by applicant. More specifically, the primer sequences taught in Borst are simply not designed for real-time PCR and differ from applicant's primers in several respects. First, the primer sequences taught in Borst amplify a sequence of 272 base pairs in length. Target sequences that are optimal for real-time fluorogenic PCR are in the range of 50-150 base pairs in length. Accordingly, applicant's primers amplify sequences of 101 and 99 base pairs in length. In addition, Borst simply does not teach primers with sequences identical to those claimed by applicant. Borst teaches an upstream primer of 5' AGCATACTGCAAGTGAAGTTG 3'and a downstream primer of 5' TTGTTGTCAACGTTAGGG 3'. These sequences are not matches with the primer sequences claimed by applicant in Claim 15. It is believed that any match found to applicant's sequences probably resulted from the fact that the entire sequence of Accession L22565 was referred to in the Borst publication. L22565 comprises the entire sequence of accession for the upstream region of the sea gene. Therefore, it may include as part of the entire sequence those potions identified by applicant as SEQ ID NO: 3 and SEQ ID NO: 5, but that does not constitute a teaching of those specific primer sequences which are only a.small part (20 or 21 bases) of the L22565 Accession. Furthermore, the majority of SEQ IDS, which are claimed and disclosed by applicant, are not included in the L22565 Accession at all since it does not include the entire sea gene. Applicant has claimed specific primers and probes of about 20 or 21 bases in length from an entire sea gene having a length of 1443 bases. Furthermore,

the Borst sequences violate several of the primer set design guidelines that must be followed to obtain a set of primer and probe oligonucleotide sequences that will perform optimally in real-time fluorogenic PCR. Specifically, the two primers described in Borst are not suitable for use in identifying the *entA* gene by real-time fluorogenic PCR for several reasons, including: (a) the amplicon being of 272 base pairs while real-time fluorogenic PCR optimally requires 50-150 base pairs; (b) Borst does not teach the melting temperature of their primer pair, and primer pairs designed for optimal performance in real- time fluorogenic PCR have melting temperatures between 58 and 60 degrees C; and (c) the downstream primer sequence taught in Borst violates the guideline for primer selection that no more than two of the five bases at the 3' end of a primer be either G or C (Borst teaches a downstream primer with three G/C bases among the five bases at the 3' end of the primer). Moreover, only one of the primers taught by Borst (that appearing in the text on p. 5423) binds a sequence inside the open reading frame encoding the SEA protein. The other primer binds a sequence upstream of the promoter for the *entA* gene. The use of this primer pair will fail to detect the gene if the gene has been excised from its native sequence and cloned behind a promoter that has been optimized for expression of the gene in another organism (as may be the case if the gene is used to create a genetically engineered biological weapon). Therefore, in addition to not being suitable for real-time PCR, the primers described on pages 5422 and 5423 do not constitute a functioning assay directed specifically and solely at sequences that encode staphylococcal enterotoxin A. It should be noted that although the Office Action indicated no claims were allowed, no rejection was included to address pending Claim 16. Claim 16 is dependent from Claim 15 and is further limiting thereto since it includes as limitations the specific sequences for the Furthermore, the Borst sequences violate several of the primer set design guidelines that must be followed to obtain a set of primer and probe oligonucleotide sequences that will perform optimally in real-time fluorogenic PCR. Specifically, the two primers described in Borst are not suitable for use in identifying the *entA* gene by real-time fluorogenic PCR for several reasons, including: (a) the amplicon being of 272 base pairs while real-time fluorogenic PCR optimally requires 50-150 base pairs; (b) Borst does not

teach the melting temperature of their primer pair, and primer pairs designed for optimal performance in real- time fluorogenic PCR have melting temperatures between 58 and 60 degrees C; and (c) the downstream primer sequence taught in Borst violates the guideline for primer selection that no more than two of the five bases at the 3' end of a primer be either G or C (Borst teaches a downstream primer with three G/C bases among the five bases at the 3' end of the primer). Moreover, only one of the primers taught by Borst (that appearing in the text on p.5423) binds a sequence inside the open reading frame encoding the SEA protein. The other primer binds a sequence upstream of the promoter for the *entA* gene. The use of this primer pair will fail to detect the gene if the gene has been excised from its native sequence and cloned behind a promoter that has been optimized for expression of the gene in another organism (as may be the case if the gene is used to create a genetically engineered biological weapon). Therefore, in addition to not being suitable for real-time PCR, the primers described on pages 5422 and 5423 do not constitute a functioning assay directed specifically and solely at sequences that encode staphylococcal enterotoxin A. It should be noted that although the Office Action indicated no claims were allowed, no rejection was included to address pending Claim 16. Claim 16 is dependent from Claim 15 and is further limiting thereto since it includes as limitations the specific sequences for the nucleic acid probe used in the method of Claim 15. Therefore, it is respectfully submitted that Claim 16 is also in condition for allowance.

In response to applicants' arguments applicants attention is brought to the claim language, claim 15 as amended is drawn to a method of determining the presence of staphylococcal enterotoxin A gene in a sample, **comprising:**

contacting a target nucleic acid sequence which **comprises a portion of the *S. aureus ent A* gene encoding staphylococcal enterotoxin A,** with polymerase chain reaction reagents specific for the target nucleic acid sequence, the polymerase chain reaction reagents including a primer selected from the group consisting of a forward primer having a specific sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4 and combinations thereof, and a reverse primer having a specific sequence

selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6 and combinations thereof, a polymerase enzyme, and a nucleic acid probe, wherein the nucleic acid probe further **comprises:**

a nucleic acid sequence that hybridizes to a portion of the target nucleic acid sequence **wherein the portion is unique to the nucleic acid encoding staphylococcal enterotoxin A;**

a reporter attached to a 5' end of the nucleic acid probe, said reporter capable of emitting a detectable signal;

a quencher attached to a 3' end of the nucleic acid probe capable of substantially quenching the reporter and prevent emission of the detectable signal, when the nucleic acid probe is intact, wherein the reporter becomes substantially unquenched when the nucleic acid probe is cleaved by the polymerase enzyme during amplification of the target nucleic acid sequence;

amplifying the target nucleic acid sequence by thermal cycling, wherein the thermal cycling will amplify the target nucleic acid sequence; and

measuring the level of fluorescence in the sample subsequent to thermal cycling, and further wherein the level of detectable signal is correlated to an amount of the nucleic acid
encoding staphylococcal enterotoxin A in the sample, **thereby quantitatively detecting the nucleic acid encoding staphylococcal enterotoxin A in the sample.**

In response to applicants' arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

It is the combination of the prior art the render claim 15 obvious. Letertre et al. teach a method of determining the presence of *staphylococcal* enterotoxin A gene in a sample using Real-Time Fluorogenic Polymerase Chain Reaction (PCR), see title and abstract. Letertre et al. teach the step of contacting a target nucleic acid sequence forming at least a portion of a nucleic acid encoding *staphylococcal* enterotoxin A, with polymerase chain reaction reagents specific for the target nucleic acid sequence, the polymerase chain reaction reagents including forward and reverse primers (see page 140, selection of primers and tables 1-3). Letertre et al. teach a nucleic acid sequence that hybridizes to a portion of the target nucleic acid sequence wherein the portion is unique to the nucleic acid encoding staphylococcal enterotoxin A (see page 140, selection of primers and tables 1-3). Letertre et al. teach a set of universal primers, FastStart Taq DNA polymerase, lighter cycle system from Roche Diagnostics and FastStart DNA Master SYBER Green in a Real –Time fluorescence PCR which covers the limitations (such a reporter attached to a 5' end of the nucleic acid probe, said reporter capable of omitting a detectable signal; and a quencher attached to a 3' end of the nucleic acid probe capable of substantially quenching the reporter and prevent omission of the detectable signal, when the nucleic acid probe is intact, wherein the reporter becomes substantially unquenched when the nucleic acid probe is cleaved by the polymerase enzyme during amplification of the target nucleic acid sequence), see page 143. Letertre et al. teach amplifying the target nucleic acid sequence by thermal cycling, wherein the thermal cycling is sufficient to amplify the target nucleic acid sequence (see page 142). Letertre et al. also teach measuring the level of fluorescence

in the sample subsequent to thermal cycling, and further wherein the level of detectable signal is correlated to an amount of the nucleic acid encoding staphylococcal enterotoxin A in the sample, thereby quantitatively detecting the nucleic acid encoding staphylococcal enterotoxin A in the sample (see page 143).

As to applicants' argument in regard to Borst et al. also teach primers and probes in the size of 20 to 21 bases in length from an entire sea gene having a length of 1443 bases (see Borst et al. pages 5422-5423 and sequence alignments for SEQ ID # 3 and SEQ ID #5).

Status of Claims

8. Claim 15 stand rejected.

Claim 16 is objected to as being dependent from rejected claim 15. Claim 16 is free of prior art.

Claims 1-9, 14 and 17-18 are withdrawn from further consideration as being drawn to non elected inventions.

Conclusion

9. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP

§ 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

I Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Khatol Shahnan-Shah whose telephone number is (571)-272-0863. The examiner can normally be reached on Mondays and Wednesdays from 12:30-6:30 PM and Thursdays from 12:30-4:30 PM. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffery Siew can be reached on 571-272-0787.

The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Khatol Shahnan-Shah
Biotechnology Patent Examiner
Art Unit 1645
August 15, 2007

JEFFREY SIEW
SUPERVISORY PATENT EXAMINER